

Supplementary information for: Molecular identification of bat fly species and associated *Bartonella* bacteria from Lopburi and Sa Kaeo Provinces in Thailand

Vadeeporn Rattananupong¹, Wachareeporn Trinachartvanit¹, Sara Bumrungsri³, Abdulloh Samoh⁴, Visut Baimai¹ & Arunee Ahantrag^{1,2*}

¹Biodiversity Research Cluster, Department of Biology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand.

²Center of Excellence for Vectors and Vector-Borne Diseases, Faculty of Science, Mahidol University, Nakhon Pathom 73170, Thailand.

³Division of Biological Science, Faculty of Science, Prince of Songkla University, Songkhla 90110, Thailand.

⁴Princess Maha Chakri Sirindhorn Natural History Museum, Prince of Songkla University, Songkhla 90110, Thailand.

*email: arunee.aha@mahidol.ac.th

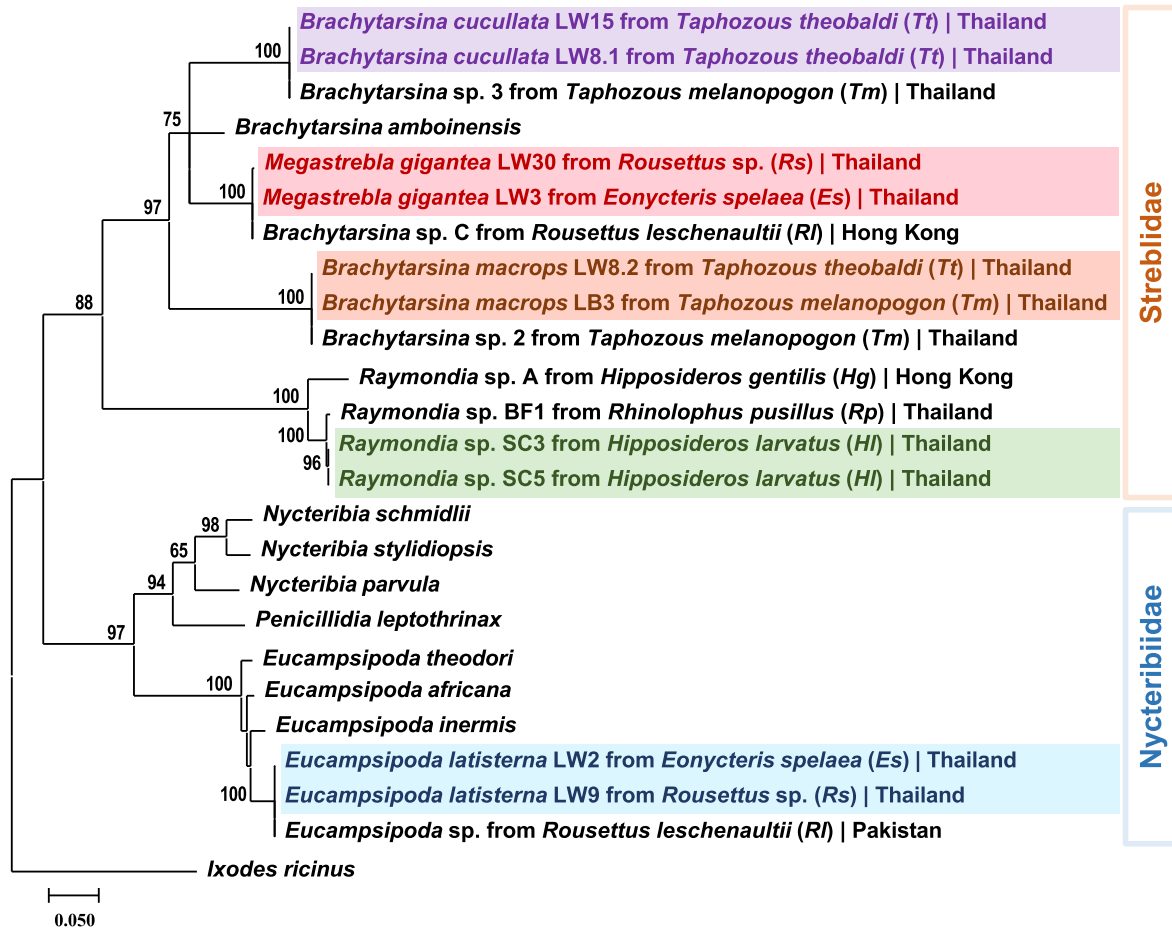


Fig. S1. Maximum likelihood phylogenetic tree of bat flies based on concatenated dataset of partial *COI*, *COII*, 16S rRNA and 18S rRNA sequences (3,117 bp). *Ixodes ricinus* was used as an outgroup for the phylogenetic analyses. Bootstrap values greater than 50% are shown above branches. Highlighted and coloured letters indicate bat fly sequences obtained in this study.

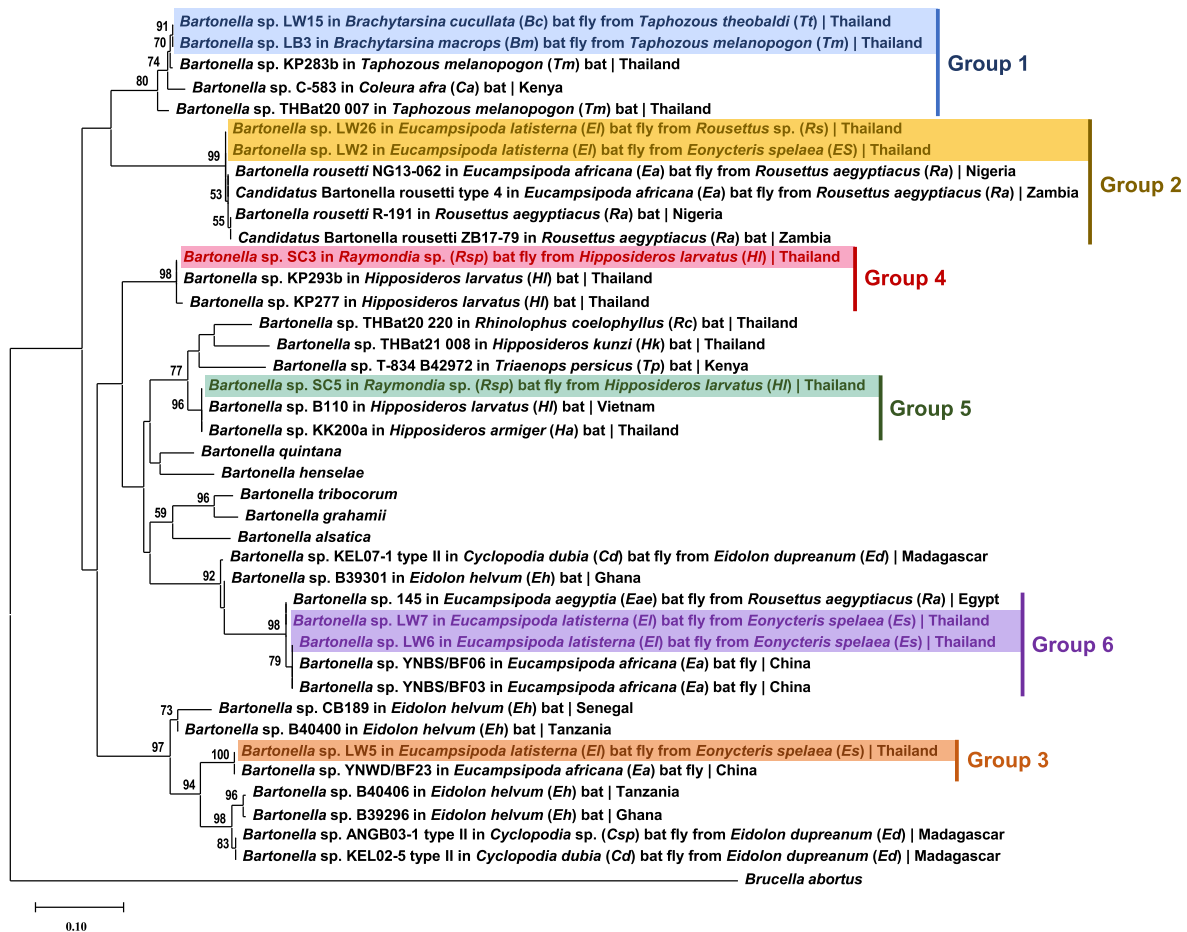


Fig. S2. Maximum likelihood phylogenetic tree of *Bartonella* spp. based on concatenated dataset of partial *gltA*, ITS, *rpoB*, *nuoG* and *ftsZ* sequences (2,211 bp). *Brucella abortus* was used as an outgroup. Bootstrap values were greater than 50% are shown above branches. Highlighted and coloured letters indicate *Bartonella* bacteria sequences obtained in this study.

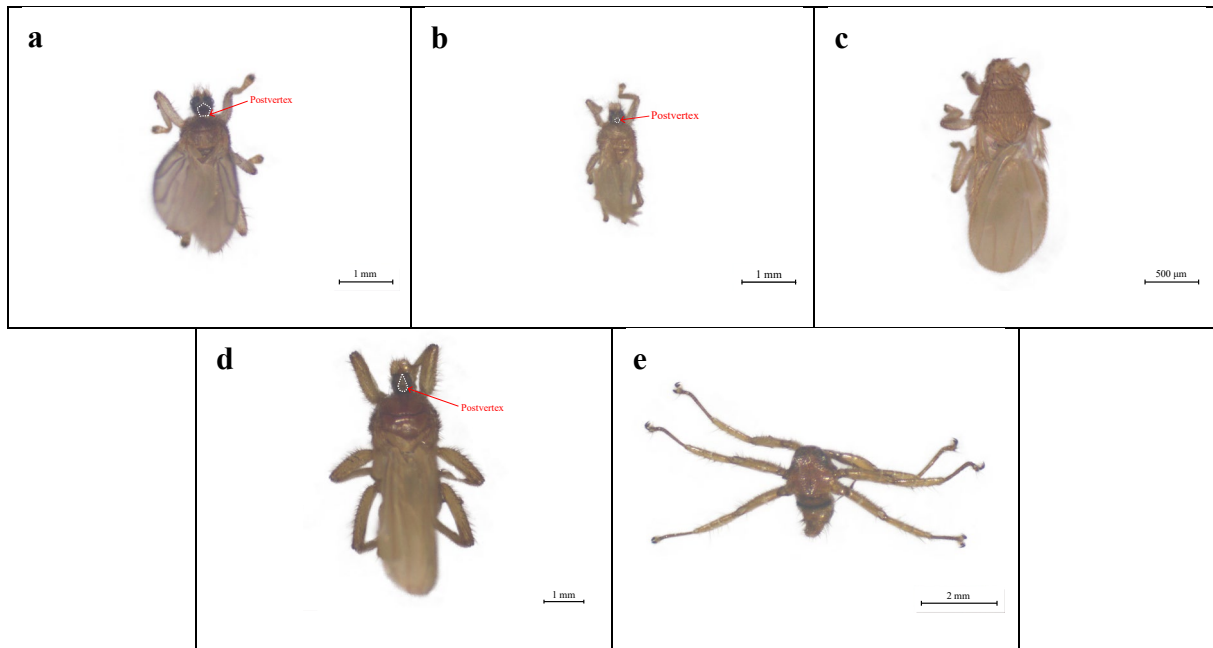


Fig. S3. Photographic images of *Brachytarsina cucullata* (a), *Brachytarsina macrops* (b), *Raymondia* sp. (c), *Megastrebla gigantea* (d) and *Eucampsipoda latisterna* (e) showing their morphological characteristics. The red arrows indicate the position of the postvertex. Scale bars are shown in each panel. (Photos: V. Rattananupong).

No.	Bat fly species	Code	Host species	Location	Gene	Accession number
1	<i>Brachytarsina cucullata</i>	LW15	<i>Taphozous theobaldi</i>	Khao Wong cave, Lopburi, Thailand	<i>COI</i>	PV477213
2	<i>Brachytarsina cucullata</i>	LW8.1	<i>Taphozous theobaldi</i>	Khao Wong cave, Lopburi, Thailand	<i>COI</i>	PV477214
3	<i>Megastrebla gigantea</i>	LW3	<i>Eonycteris spelaea</i>	Khao Wong cave, Lopburi, Thailand	<i>COI</i>	PV477215
4	<i>Megastrebla gigantea</i>	LW30	<i>Rousettus</i> sp.	Khao Wong cave, Lopburi, Thailand	<i>COI</i>	PV477216
5	<i>Brachytarsina macrops</i>	LW8.2	<i>Taphozous theobaldi</i>	Khao Wong cave, Lopburi, Thailand	<i>COI</i>	PV477217
6	<i>Brachytarsina macrops</i>	LB3	<i>Taphozous melanopogon</i>	Bot Yawas cave, Lopburi, Thailand	<i>COI</i>	PV477218
7	<i>Raymondia</i> sp.	SC5	<i>Hipposideros larvatus</i>	Khao Chakan cave, Sa Kao, Thailand	<i>COI</i>	PV477219
8	<i>Raymondia</i> sp.	SC3	<i>Hipposideros larvatus</i>	Khao Chakan cave, Sa Kao, Thailand	<i>COI</i>	PV477220
9	<i>Eucampsipoda latisterna</i>	LW2	<i>Eonycteris spelaea</i>	Khao Wong cave, Lopburi, Thailand	<i>COI</i>	PV477221
10	<i>Eucampsipoda latisterna</i>	LW9	<i>Rousettus</i> sp.	Khao Wong cave, Lopburi, Thailand	<i>COI</i>	PV477222
11	<i>Brachytarsina cucullata</i>	LW15	<i>Taphozous theobaldi</i>	Khao Wong cave, Lopburi, Thailand	<i>COII</i>	PV491571
12	<i>Brachytarsina cucullata</i>	LW8.1	<i>Taphozous theobaldi</i>	Khao Wong cave, Lopburi, Thailand	<i>COII</i>	PV491572
13	<i>Megastrebla gigantea</i>	LW3	<i>Eonycteris spelaea</i>	Khao Wong cave, Lopburi, Thailand	<i>COII</i>	PV491573
14	<i>Megastrebla gigantea</i>	LW30	<i>Rousettus</i> sp.	Khao Wong cave, Lopburi, Thailand	<i>COII</i>	PV491574
15	<i>Brachytarsina macrops</i>	LW8.2	<i>Taphozous theobaldi</i>	Khao Wong cave, Lopburi, Thailand	<i>COII</i>	PV491575
16	<i>Brachytarsina macrops</i>	LB3	<i>Taphozous melanopogon</i>	Bot Yawas cave, Lopburi, Thailand	<i>COII</i>	PV491576
17	<i>Raymondia</i> sp.	SC5	<i>Hipposideros larvatus</i>	Khao Chakan cave, Sa Kao, Thailand	<i>COII</i>	PV491577
18	<i>Raymondia</i> sp.	SC3	<i>Hipposideros larvatus</i>	Khao Chakan cave, Sa Kao, Thailand	<i>COII</i>	PV491578
19	<i>Eucampsipoda latisterna</i>	LW2	<i>Eonycteris spelaea</i>	Khao Wong cave, Lopburi, Thailand	<i>COII</i>	PV491579
20	<i>Eucampsipoda latisterna</i>	LW9	<i>Rousettus</i> sp.	Khao Wong cave, Lopburi, Thailand	<i>COII</i>	PV491580
21	<i>Brachytarsina cucullata</i>	LW15	<i>Taphozous theobaldi</i>	Khao Wong cave, Lopburi, Thailand	16S rRNA	PV463885
22	<i>Brachytarsina cucullata</i>	LW8.1	<i>Taphozous theobaldi</i>	Khao Wong cave, Lopburi, Thailand	16S rRNA	PV463886
23	<i>Megastrebla gigantea</i>	LW3	<i>Eonycteris spelaea</i>	Khao Wong cave, Lopburi, Thailand	16S rRNA	PV463887
24	<i>Megastrebla gigantea</i>	LW30	<i>Rousettus</i> sp.	Khao Wong cave, Lopburi, Thailand	16S rRNA	PV463888
25	<i>Brachytarsina macrops</i>	LW8.2	<i>Taphozous theobaldi</i>	Khao Wong cave, Lopburi, Thailand	16S rRNA	PV463889
26	<i>Brachytarsina macrops</i>	LB3	<i>Taphozous melanopogon</i>	Bot Yawas cave, Lopburi, Thailand	16S rRNA	PV463890
27	<i>Raymondia</i> sp.	SC5	<i>Hipposideros larvatus</i>	Khao Chakan cave, Sa Kao, Thailand	16S rRNA	PV463891
28	<i>Raymondia</i> sp.	SC3	<i>Hipposideros larvatus</i>	Khao Chakan cave, Sa Kao, Thailand	16S rRNA	PV463892
29	<i>Eucampsipoda latisterna</i>	LW2	<i>Eonycteris spelaea</i>	Khao Wong cave, Lopburi, Thailand	16S rRNA	PV463893
30	<i>Eucampsipoda latisterna</i>	LW9	<i>Rousettus</i> sp.	Khao Wong cave, Lopburi, Thailand	16S rRNA	PV463894
31	<i>Brachytarsina cucullata</i>	LW15	<i>Taphozous theobaldi</i>	Khao Wong cave, Lopburi, Thailand	18S rRNA	PV470912
32	<i>Brachytarsina cucullata</i>	LW8.1	<i>Taphozous theobaldi</i>	Khao Wong cave, Lopburi, Thailand	18S rRNA	PV470913
33	<i>Megastrebla gigantea</i>	LW3	<i>Eonycteris spelaea</i>	Khao Wong cave, Lopburi, Thailand	18S rRNA	PV470914

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70 **Table S1.** Accession number of bat fly species in this study.

No.	Bat fly species	Code	Host species	Location	Gene	Accession number
34	<i>Megastrebla gigantea</i>	LW30	<i>Rousettus</i> sp.	Khao Wong cave, Lopburi, Thailand	18S rRNA	PV470915
35	<i>Brachytarsina macrops</i>	LW8.2	<i>Taphozous theobaldi</i>	Khao Wong cave, Lopburi, Thailand	18S rRNA	PV470916
36	<i>Brachytarsina macrops</i>	LB3	<i>Taphozous melanopogon</i>	Bot Yawas cave, Lopburi, Thailand	18S rRNA	PV470917
37	<i>Raymondia</i> sp.	SC5	<i>Hipposideros larvatus</i>	Khao Chakan cave, Sa Kaeo, Thailand	18S rRNA	PV470918
38	<i>Raymondia</i> sp.	SC3	<i>Hipposideros larvatus</i>	Khao Chakan cave, Sa Kaeo, Thailand	18S rRNA	PV470919
39	<i>Eucampsipoda latisterna</i>	LW2	<i>Eonycteris spelaea</i>	Khao Wong cave, Lopburi, Thailand	18S rRNA	PV470920
40	<i>Eucampsipoda latisterna</i>	LW9	<i>Rousettus</i> sp.	Khao Wong cave, Lopburi, Thailand	18S rRNA	PV470921

Table S1. (continued)

No.	<i>Bartonella</i> spp.	Code	Bat fly species	Host species	Location	Gene	Accession number
1	<i>Bartonella</i> sp.	LW15	<i>Brachytarsina cucullata</i>	<i>Taphozous theobaldi</i>	Khao Wong cave, Lopburi, Thailand	<i>gltA</i>	PV491581
2	<i>Bartonella</i> sp.	LB3	<i>Brachytarsina macrops</i>	<i>Taphozous melanopogon</i>	Bot Yawas cave, Lopburi, Thailand	<i>gltA</i>	PV491582
3	<i>Bartonella</i> sp.	LW2	<i>Eucampsipoda latisterna</i>	<i>Eonycteris spelaea</i>	Khao Wong cave, Lopburi, Thailand	<i>gltA</i>	PV491583
4	<i>Bartonella</i> sp.	LW26	<i>Eucampsipoda latisterna</i>	<i>Rousettus</i> sp.	Khao Wong cave, Lopburi, Thailand	<i>gltA</i>	PV491584
5	<i>Bartonella</i> sp.	SC3	<i>Raymondia</i> sp.	<i>Hipposideros larvatus</i>	Khao Chakan cave, Sa Kaeo, Thailand	<i>gltA</i>	PV491585
6	<i>Bartonella</i> sp.	SC5	<i>Raymondia</i> sp.	<i>Hipposideros larvatus</i>	Khao Chakan cave, Sa Kaeo, Thailand	<i>gltA</i>	PV491586
7	<i>Bartonella</i> sp.	LW5	<i>Eucampsipoda latisterna</i>	<i>Eonycteris spelaea</i>	Khao Wong cave, Lopburi, Thailand	<i>gltA</i>	PV491587
8	<i>Bartonella</i> sp.	LW6	<i>Eucampsipoda latisterna</i>	<i>Eonycteris spelaea</i>	Khao Wong cave, Lopburi, Thailand	<i>gltA</i>	PV491588
9	<i>Bartonella</i> sp.	LW7	<i>Eucampsipoda latisterna</i>	<i>Eonycteris spelaea</i>	Khao Wong cave, Lopburi, Thailand	<i>gltA</i>	PV491589
10	<i>Bartonella</i> sp.	LW15	<i>Brachytarsina cucullata</i>	<i>Taphozous theobaldi</i>	Khao Wong cave, Lopburi, Thailand	<i>rpoB</i>	PV491590
11	<i>Bartonella</i> sp.	LB3	<i>Brachytarsina macrops</i>	<i>Taphozous melanopogon</i>	Bot Yawas cave, Lopburi, Thailand	<i>rpoB</i>	PV491591
12	<i>Bartonella</i> sp.	LW26	<i>Eucampsipoda latisterna</i>	<i>Rousettus</i> sp.	Khao Wong cave, Lopburi, Thailand	<i>rpoB</i>	PV491592
13	<i>Bartonella</i> sp.	SC3	<i>Raymondia</i> sp.	<i>Hipposideros larvatus</i>	Khao Chakan cave, Sa Kaeo, Thailand	<i>rpoB</i>	PV491593
14	<i>Bartonella</i> sp.	SC5	<i>Raymondia</i> sp.	<i>Hipposideros larvatus</i>	Khao Chakan cave, Sa Kaeo, Thailand	<i>rpoB</i>	PV491594
15	<i>Bartonella</i> sp.	LW5	<i>Eucampsipoda latisterna</i>	<i>Eonycteris spelaea</i>	Khao Wong cave, Lopburi, Thailand	<i>rpoB</i>	PV491595
16	<i>Bartonella</i> sp.	LW6	<i>Eucampsipoda latisterna</i>	<i>Eonycteris spelaea</i>	Khao Wong cave, Lopburi, Thailand	<i>rpoB</i>	PV491596
17	<i>Bartonella</i> sp.	LW7	<i>Eucampsipoda latisterna</i>	<i>Eonycteris spelaea</i>	Khao Wong cave, Lopburi, Thailand	<i>rpoB</i>	PV491597
18	<i>Bartonella</i> sp.	LW15	<i>Brachytarsina cucullata</i>	<i>Taphozous theobaldi</i>	Khao Wong cave, Lopburi, Thailand	<i>nuoG</i>	PV491598
19	<i>Bartonella</i> sp.	LB3	<i>Brachytarsina macrops</i>	<i>Taphozous melanopogon</i>	Bot Yawas cave, Lopburi, Thailand	<i>nuoG</i>	PV491599
20	<i>Bartonella</i> sp.	LW26	<i>Eucampsipoda latisterna</i>	<i>Rousettus</i> sp.	Khao Wong cave, Lopburi, Thailand	<i>nuoG</i>	PV491600
21	<i>Bartonella</i> sp.	SC3	<i>Raymondia</i> sp.	<i>Hipposideros larvatus</i>	Khao Chakan cave, Sa Kaeo, Thailand	<i>nuoG</i>	PV491601
22	<i>Bartonella</i> sp.	SC5	<i>Raymondia</i> sp.	<i>Hipposideros larvatus</i>	Khao Chakan cave, Sa Kaeo, Thailand	<i>nuoG</i>	PV491602
23	<i>Bartonella</i> sp.	LW5	<i>Eucampsipoda latisterna</i>	<i>Eonycteris spelaea</i>	Khao Wong cave, Lopburi, Thailand	<i>nuoG</i>	PV491603
24	<i>Bartonella</i> sp.	LW6	<i>Eucampsipoda latisterna</i>	<i>Eonycteris spelaea</i>	Khao Wong cave, Lopburi, Thailand	<i>nuoG</i>	PV491604
25	<i>Bartonella</i> sp.	LW7	<i>Eucampsipoda latisterna</i>	<i>Eonycteris spelaea</i>	Khao Wong cave, Lopburi, Thailand	<i>nuoG</i>	PV491605
26	<i>Bartonella</i> sp.	LW15	<i>Brachytarsina cucullata</i>	<i>Taphozous theobaldi</i>	Khao Wong cave, Lopburi, Thailand	<i>ftsZ</i>	PV491606
27	<i>Bartonella</i> sp.	LB3	<i>Brachytarsina macrops</i>	<i>Taphozous melanopogon</i>	Bot Yawas cave, Lopburi, Thailand	<i>ftsZ</i>	PV491607
28	<i>Bartonella</i> sp.	SC5	<i>Raymondia</i> sp.	<i>Hipposideros larvatus</i>	Khao Chakan cave, Sa Kaeo, Thailand	<i>ftsZ</i>	PV491608
29	<i>Bartonella</i> sp.	LW6	<i>Eucampsipoda latisterna</i>	<i>Eonycteris spelaea</i>	Khao Wong cave, Lopburi, Thailand	<i>ftsZ</i>	PV491609
30	<i>Bartonella</i> sp.	LW7	<i>Eucampsipoda latisterna</i>	<i>Eonycteris spelaea</i>	Khao Wong cave, Lopburi, Thailand	<i>ftsZ</i>	PV491610
31	<i>Bartonella</i> sp.	LW15	<i>Brachytarsina cucullata</i>	<i>Taphozous theobaldi</i>	Khao Wong cave, Lopburi, Thailand	ITS region	PV501196
32	<i>Bartonella</i> sp.	LB3	<i>Brachytarsina macrops</i>	<i>Taphozous melanopogon</i>	Bot Yawas cave, Lopburi, Thailand	ITS region	PV501197
33	<i>Bartonella</i> sp.	LW2	<i>Eucampsipoda latisterna</i>	<i>Eonycteris spelaea</i>	Khao Wong cave, Lopburi, Thailand	ITS region	PV501198

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92 **Table S2.** Accession number of *Bartonella* spp. in this study.

No.	<i>Bartonella</i> spp.	Code	Bat fly species	Host species	Location	Gene	Accession number
34	<i>Bartonella</i> sp.	LW26	<i>Eucampsipoda latisterna</i>	<i>Rousettus</i> sp.	Khao Wong cave, Lopburi, Thailand	ITS region	PV501199
35	<i>Bartonella</i> sp.	SC3	<i>Raymondia</i> sp.	<i>Hipposideros larvatus</i>	Khao Chakan cave, Sa Kaeo, Thailand	ITS region	PV501200
36	<i>Bartonella</i> sp.	SC5	<i>Raymondia</i> sp.	<i>Hipposideros larvatus</i>	Khao Chakan cave, Sa Kaeo, Thailand	ITS region	PV501201
37	<i>Bartonella</i> sp.	LW5	<i>Eucampsipoda latisterna</i>	<i>Eonycteris spelaea</i>	Khao Wong cave, Lopburi, Thailand	ITS region	PV501202
38	<i>Bartonella</i> sp.	LW6	<i>Eucampsipoda latisterna</i>	<i>Eonycteris spelaea</i>	Khao Wong cave, Lopburi, Thailand	ITS region	PV501203
39	<i>Bartonella</i> sp.	LW7	<i>Eucampsipoda latisterna</i>	<i>Eonycteris spelaea</i>	Khao Wong cave, Lopburi, Thailand	ITS region	PV501204

Table S2. (continued)

Gene/Species		Bat fly species and % identity				
		<i>B. cucullata</i> LW15	<i>B. macrops</i> LB3	<i>M. gigantea</i> LW3	<i>Raymondia</i> sp. SC5	<i>E. latisterna</i> LW9
<i>COI</i>	<i>B. cucullata</i> LW15		85.08	87.70	81.15	80.23
	<i>B. macrops</i> LB3			87.05	81.12	80.81
	<i>M. gigantea</i> LW3				81.97	80.23
	<i>Raymondia</i> sp. SC5					78.79
	<i>E. latisterna</i> LW9					
<i>COII</i>	<i>B. cucullata</i> LW15		87.58	88.57	83.11	82.83
	<i>B. macrops</i> LB3			88.40	81.94	83.50
	<i>M. gigantea</i> LW3				84.09	83.72
	<i>Raymondia</i> sp. SC5					79.93
	<i>E. latisterna</i> LW9					
16S rRNA	<i>B. cucullata</i> LW15		89.59	90.24	83.37	85.09
	<i>B. macrops</i> LB3			90.56	82.52	82.97
	<i>M. gigantea</i> LW3				84.20	85.82
	<i>Raymondia</i> sp. SC5					79.76
	<i>E. latisterna</i> LW9					
18S rRNA	<i>B. cucullata</i> LW15		97.12	96.22	95.86	92.60
	<i>B. macrops</i> LB3			98.24	97.68	93.08
	<i>M. gigantea</i> LW3				96.91	92.73
	<i>Raymondia</i> sp. SC5					93.15
	<i>E. latisterna</i> LW9					

Table S3. Pairwise comparison of gene sequence identity (%) among five species of bat flies based on *COI*, *COII*, 16S rRNA and 18S rRNA genes.

Species Gene/Species		Bartonella species Groups and % identity					
		Group 1 (sp. LW15)	Group 2 (sp. LW26)	Group 3 (sp. LW5)	Group 4 (sp. SC3)	Group 5 (sp. SC5)	Group 6 (sp. LW7)
<i>gltA</i>	Group 1 (sp. LW15)		86.14	84.49	88.78	85.48	85.81
	Group 2 (sp. LW26)			84.16	85.81	86.14	83.83
	Group 3 (sp. LW5)				86.14	85.81	85.48
	Group 4 (sp. SC3)					89.77	86.80
	Group 5 (sp. SC5)						86.47
	Group 6 (sp. LW7)						
ITS	Group 1 (sp. LW15)		79.00	79.42	69.47	67.71	65.81
	Group 2 (sp. LW26)			75.52	68.51	67.60	66.80
	Group 3 (sp. LW5)				67.42	71.38	64.80
	Group 4 (sp. SC3)					68.06	65.10
	Group 5 (sp. SC5)						66.67
	Group 6 (sp. LW7)						
<i>rpoB</i>	Group 1 (sp. LW15)		86.84	87.34	89.87	88.35	88.10
	Group 2 (sp. LW26)			84.05	84.30	85.82	83.54
	Group 3 (sp. LW5)				87.34	87.88	87.09
	Group 4 (sp. SC3)					89.87	88.61
	Group 5 (sp. SC5)						87.09
	Group 6 (sp. LW7)						
<i>nuoG</i>	Group 1 (sp. LW15)		84.30	87.71	84.98	86.02	87.03
	Group 2 (sp. LW26)			86.01	83.96	86.49	87.03
	Group 3 (sp. LW5)				89.08	80.58	88.40
	Group 4 (sp. SC3)					89.19	87.03
	Group 5 (sp. SC5)						87.57
	Group 6 (sp. LW7)						
<i>ftsZ</i>	Group 1 (sp. LW15)		NA	NA	NA	73.84	70.31
	Group 2 (sp. LW26)			NA	NA	NA	NA
	Group 3 (sp. LW5)				NA	NA	NA
	Group 4 (sp. SC3)					NA	NA
	Group 5 (sp. SC5)						71.63
	Group 6 (sp. LW7)						

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127 **Table S4.** Pairwise comparison of gene sequence identity (%) among six *Bartonella* species

128 Groups based on *gltA*, *rpoB*, *nuoG*, *ftsZ* genes and ITS region. NA indicates data not available.